Induction of unnatural immunity: prospects for a broadly protective universal influenza vaccine

Gary J Nabel & Anthony S Fauci

The immune system normally responds to influenza virus by making neutralizing antibodies to regions of the viral spike, the hemagglutinin, that vary year to year. This natural response protects against circulating subtypes but necessitates production of new vaccines annually. Newer vaccine approaches have succeeded in eliciting broadly neutralizing antibodies to highly conserved yet vulnerable regions of the hemagglutinin and suggest potential pathways for the development of universal influenza vaccines.

In the struggle between viral infection and immune protection, influenza viruses have perfected the art of evasion. As humans develop immunity to the current circulating strains, the virus evolves variants through genetic mutation, leading to antigenic drift in humans or other influenza-susceptible species. These new strains evade neutralization and give rise to new seasonal influenza viruses that claim the lives of more than 250,000 people worldwide each year. Current influenza vaccination campaigns rely on a well-honed process: every year, a new vaccine is prepared that aims to match the strains predicted to circulate in the coming flu season. For more than 65 years, this pragmatic approach has saved lives and benefitted public health. Yet the preparation of new influenza vaccines costs $2–4 billion yearly. In addition, a vaccine prepared for an upcoming influenza season does not always completely match the actual strains that circulate in that season. More importantly, as witnessed in the 2009 H1N1 outbreak, completely new strains can unexpectedly emerge against which contemporary vaccines provide little or no protection. The emergence of completely new strains is potentially devastating, because most of the world's population lacks adequate background immunity that might have come from either natural exposure or vaccination. This potential vulnerability underscores the need to advance a universal influenza vaccine as a means to address a serious public health concern.

The strategic approach to influenza vaccine development is typical of all licensed vaccines: the aim is to mimic natural exposure to the virus using inactivated or attenuated viruses that provoke immune recognition without causing disease. Protection is conferred by the antibody response that neutralizes the influenza virus, measured by the hemagglutination inhibition assay. This laboratory test serves as the basis for predicting the efficacy of new vaccines and for licensing them. However, with influenza, the process of vaccine development needs to be repeated annually, as influenza outbreaks predictably occur each year and viruses usually drift or change their hemagglutinin (HA) enough from year to year that infection or vaccination with strains from a particular year often does not provide adequate protection against those circulating in the subsequent year. An enigma for the field of influenza vaccinology is the fact that despite repeated exposures to influenza, most humans do not ultimately develop universal protection against any emerging influenza strain. One potential explanation for this is that in natural infection, the virus does not readily expose to the host immune system those components of its structure that do not vary from strain to strain, because immune responses to such components would probably protect against drifting strains. Hence, the predominant components of the virus that the immune system adequately sees upon infection are those that change with each emerging strain, reducing the likelihood of universal protection induced by prior infection. Because currently employed vaccination strategies use killed or attenuated influenza viruses that mimic those causing natural infection, the same constraints hold true for influenza vaccination.

Two areas of investigation have emerged that provide opportunities to improve upon the traditional approach to influenza vaccination. They are based on understanding natural immunity to influenza and the structure of the viral HA. Some individuals are immune to subtypes of influenza virus to which they have not previously been exposed through natural infection or immunization. This protection, termed heterosubtypic immunity, suggests that regions of the virus shared by different strains can be recognized by the immune system[1–3]. These common regions of vulnerability that serve as the basis for natural cross-protective immunity have not been well defined. It has been proposed that certain components of either HA, the nucleoprotein (NP) or M2 proteins of the virus may be the targets for heterosubtypic immunity (reviewed in ref. 4). Indeed, some vaccine efforts have aimed to elicit antiviral $CD8^+$ T cell responses by gene-based immunization with the highly conserved NP and M2 proteins.

A possible explanation for cross-protective immunity has arisen from an understanding of the molecular targets of broadly neutralizing monoclonal antibodies directed to the viral

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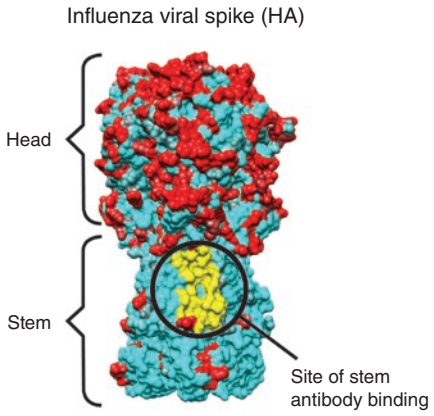

Figure 1 Molecular model of the influenza virus spike and the site of the highly conserved region of vulnerability in the stem region. The spike, composed of a trimeric HA, consists of an upper (head) and lower (stem) region that mediates attachment and entry of influenza into cells of the respiratory tract. The sites of amino acid variability among influenza strains (red, <98% conserved) and the location of the highly conserved stem antibody site (yellow) are highlighted.

spike, which mediates attachment of the virus to specific cellular receptors and promotes entry of the virus into the target cell. The spike is composed of a trimer of the viral HA protein and contains a head and a stem region. Although there is considerable diversity in the amino acid sequences of the HA protein of different influenza viruses, a specific region of the stem is conserved among many viral strains (**Fig. 1**). The sixteen different HAs found in animal and human influenza A viruses can be classified broadly into two groups (groups 1 and 2) on the basis of phylogenetic sequence analysis (**Fig. 2**; see also ref. 5). Monoclonal antibodies directed to this HA stem region of the group 1 influenza HA strains recognize a variety of strains, including different subtypes within the group, and mediate neutralization of many viruses within that group[6–12]. A number of studies have suggested the possibility of eliciting such antibodies through immunization, either to conserved regions of specific subtypes or across subtypes[12–15]. Recently, it has been possible to stimulate the production of stem-targeting, cross-neutralizing antibodies in several species, including nonhuman primates, by vaccination, and protection has been shown in both mice and ferrets, proving that nonhuman primates and possibly humans are capable of mounting such protective responses. This raises the possibility of a new approach to influenza vaccination by targeting cross-protective shared HA stem epitopes and inducing immune responses of sufficient magnitude to provide broad protection[16].

On the basis of these insights, one might envision two paths that could lead to development and licensure of universal influenza vaccines. The strategy for advancement is likely to affect the speed and likelihood for success of such a human vaccine. The first approach is to elicit antibodies to the stem region that cross-react with HAs within each of the group 1 or 2 viruses (**Fig. 2**). Because it is unknown whether the human immune system will be able to generate protective antibodies of sufficiently high titer to all members within each group, a second possibility would be to stimulate cross-neutralizing antibody responses to selected subtypes of influenza, specifically those that historically are most likely to cause disease in humans. A recent study using vaccination to elicit these antibodies in several animal models demonstrated such cross-reactivity between subtypes[16]. For example, HA priming with plasmid DNA followed by a seasonal vaccine or HA-adenoviral vector boosting can stimulate production of antibodies that cross-neutralize not only H1N1 viruses but also H2N2 or H5N1 viruses[16]. The most effective neutralization, however, is directed to isolates within the same subtype, H1N1 (ref. 16). A complementary strategy has been described by vaccination with 'headless' HA immunogens, which might also achieve heterosubtypic cross-neutralization, although it has not yet been shown to elicit broadly neutralizing stem-directed antibodies[13]. In either case, the subtype strategy would involve the development of a composite vaccine that would provide coverage for all variants within the H1, H2 and H3 strains, that is, first-tier subtypes that are known to cause pandemics in humans (**Fig. 3**). In addition, a next-generation vaccine could broaden the response to other circulating strains that pose a higher risk for pandemic infection in humans on the basis of their prevalence in animal reservoirs and ability to occasionally infect humans, such as the highly pathogenic avian influenza virus, H5N1, and other high-risk strains, such as H9 in group 1 and H7 in group 2. Vaccines that elicit cross-protective stem-specific antibodies to these second-tier strains could be then advanced into development as success is achieved with first-tier strains and incorporated as secondary vaccines that may eventually be co-administered.

In addition to the stem-directed universal flu vaccine antibodies, a number of studies are aimed at the development of T cell–based vaccines against highly conserved viral proteins such as the NP or M2. Animal studies suggest that this approach is less effective in protecting against infection with influenza virus[17]. This mode of protection would most likely require use in combination with HA-directed antibody vaccines, although their ultimate potential for efficacy in humans is uncertain. Similarly, efforts have been made to stimulate an antibody response to conserved regions of the viral M2 or neuraminidase (NA) proteins (reviewed in refs. 4,18). Although these targets are also worthy of exploration, antibodies directed to them do not neutralize virus, making this approach more problematic.

As mentioned above, the traditional approach to vaccine development takes advantage of the natural immune response to viral infection. In the case of a universal influenza vaccine, the goal is to elicit a response to the virus that does not occur naturally. There are several reasons why it may be possible to generate 'unnatural' immunity to influenza virus. First, the major immunodominant region of the influenza virus

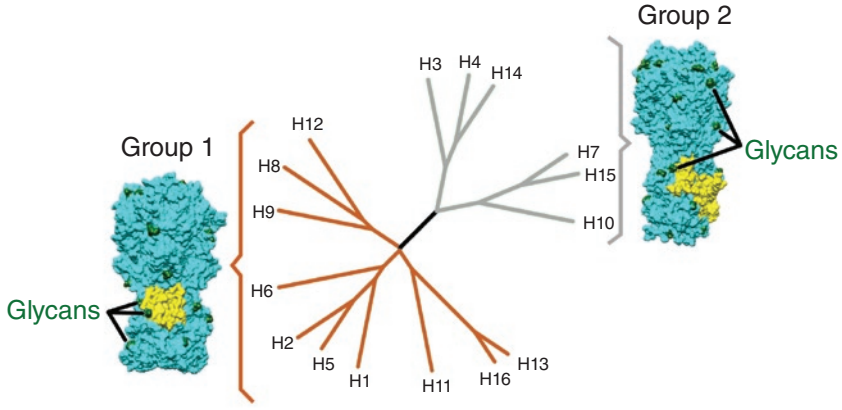

Figure 2 Phylogenetic classification of influenza HAs. Genetic relatedness of the 16 diverse HAs suggests that there are two broad groups of viruses composed of the indicated HAs. Each group seems to share a region of sequence conservation in the stem that can serve as a target of broadly neutralizing antibodies directed to this region. This scheme has been modified from one described in a previous report[5]. Conserved sites for group 1 and group 2 viruses are highlighted in yellow, on the basis of H1N1 and H3N2 sequence analysis and the known site of antibody interaction in H1N1 by structural analysis[8,9]. Conserved glycosylation sites adjacent to the stem region are shown in green.

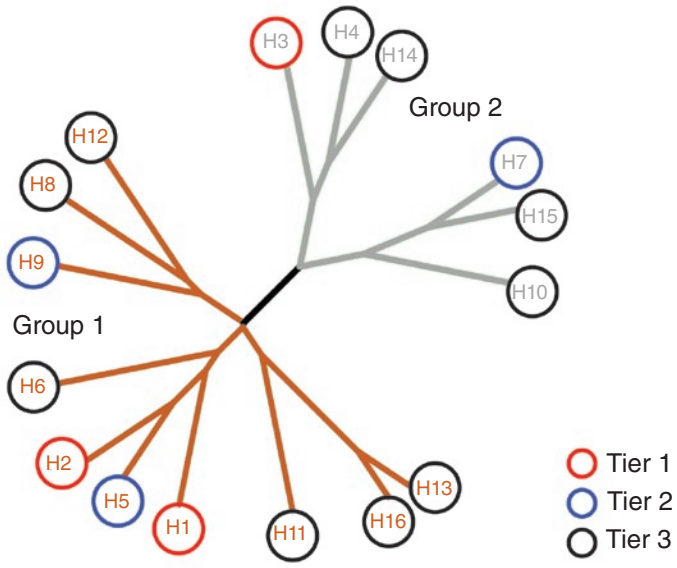

Figure 3 Hierarchies of influenza strains and a possible alternative prioritization for influenza vaccine development in humans. Three tiers of viruses, on the basis of their likelihood of causing widespread disease in humans, can be targeted for strain-specific vaccines that could recognize diverse viruses, giving rise to seasonal vaccines and eventually providing protection against new pandemic viruses.

resides on the head of the viral spike, which is multivalent and highly exposed. The stem region of the spike found at the trimeric base of the HA is surrounded by adjacent HAs on the surface of the virus as well as NA, which may shield the stem from recognition by the immune system and hence interfere with the generation of a natural immune response against it. Yet recent studies have shown that stem-targeting antibodies induced by vaccination can protect against viral infection in mice and ferrets[16], suggesting that this region is not conformationally inaccessible and can be reached by the antibodies once they are generated. Thus, the issue is not whether these antibodies can bind these crucial sites, but what is the most effective way to induce and maintain them. Next-generation vaccines can elicit these antibodies by processes that do not occur naturally during viral infection. For example, a more potent T cell immune response against the viral HA would provide a stronger stimulus that helps B cells to synthesize antibodies. This effect could be achieved in several ways. One example would be to use adjuvants that stimulate increased T cell help in seasonal influenza vaccines, one of several alternative solutions to the problem[19]. In addition, the delivery of the viral HA by a gene-based vector such as a DNA vaccine allows expression of HA in the absence of other viral proteins that may mask its presentation to the immune system, thus providing a stimulus that otherwise cannot engage this region of the molecule[16]. Finally, better understanding of the molecular nature of this target will undoubtedly lead to structural and genetic approaches to the development of new immunogens that focus on this site. The recently described broadly neutralizing monoclonal antibodies and vaccine-induced antisera can guide the development of structure-based, rationally designed proteins that contain only the relevant regions of the HA. First-generation immunogens have provided an indication that this approach may be successful[16], and others are in development[13]; however, much work needs to be done to achieve the goal of universal influenza vaccination.

Taken together, the recent advances in understanding heterosubtypic immunity, common structural determinants on HA and the generation of broadly neutralizing immune responses by prime-boost vaccination suggest that the goal of a universal cross-protective influenza vaccine is feasible. New tools of gene-based vaccination, immunologic adjuvants and monoclonal antibodies that facilitate structure-based vaccine design can aid in the development of vaccine candidates that induce an immune response to the highly conserved structural domains shared among diverse viruses. In essence, it may be possible for a new generation of vaccines to do even better than natural infection in eliciting a safe and effective immune response against the ever-present threat of influenza.

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1. Schulman, J.L. & Kilbourne, E.D. *J. Bacteriol.* **89**, 170–174 (1965).
2. Mbawuike, I.N., Six, H.R., Cate, T.R. & Couch, R.B. *J. Virol.* **64**, 1370–1374 (1990).
3. Epstein, S.L. *et al. J. Immunol.* **158**, 1222–1230 (1997).
4. Roose, K., Fiers, W. & Saelens, X. *Drug News Perspect.* **22**, 80–92 (2009).
5. Russell, R.J. *et al. Proc. Natl. Acad. Sci. USA* **105**, 17736–17741 (2008).
6. Corti, D. *et al. J. Clin. Invest.* **120**, 1663–1673 (2010).
7. Ekiert, D.C. *et al. Science* **324**, 246–251 (2009).
8. Kashyap, A.K. *et al. Proc. Natl. Acad. Sci. USA* **105**, 5986–5991 (2008).
9. Kwong, P.D. & Wilson, I.A. *Nat. Immunol.* **10**, 573–578 (2009).
10. Okuno, Y., Isegawa, Y., Sasao, F. & Ueda, S. *J. Virol.* **67**, 2552–2558 (1993).
11. Sui, J. *et al. Nat. Struct. Mol. Biol.* **16**, 265–273 (2009).
12. Wang, T.T. *et al. PLoS Pathog.* **6**, e1000796 (2010).
13. Steel, J. *et al. MBio.* **1**, e00018-10 (2010).
14. Kashyap, A.K. *et al. PLoS Pathog.* **6**, e1000990 (2010).
15. Bommakanti, G. *et al. Proc. Natl. Acad. Sci. USA* **107**, 13701–13706 (2010).
16. Wei, C.J. *et al. Science* **329**, 1060–1064 (2010).
17. Rao, S.S. *et al. PLoS ONE* **5**, e9812 (2010).
18. Gerhard, W., Mozdzanowska, K. & Zharikova, D. *Emerg. Infect. Dis.* **12**, 569–574 (2006).
19. Khurana, S. *et al. Sci. Transl. Med.* **2**, 15ra5 (2010).